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TEXTURE ANALYSIS PARAMETERS AS WINEGRAPES VARIETAL MARKERS AND RIPENESS PREDICTORS

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ABSTRACT

The texture parameters of three red grape varieties (Mencía, Brancellao and Merenzao) cultivated in Galician vineyards (Northwest Spain) were determined. Different ripening stages (A: 176 ± 8 g/l reducing sugars; B: 193 ± 8 g/l reducing sugars; C: 210 ± 8 g/l reducing sugars; D: 227 ± 8 g/l reducing sugars) were also considered. Principal component analysis (PCA) was performed for a better understanding of the differences found among grapes according to variety and ripening stage based on the skin and berry texture parameters. The parameters differentiating varieties were the skin break force and energy measured on the lateral side, whereas ripening stages can be classified on the basis of berry cohesiveness. The hardest berry skin was associated with Merenzao variety with skin break force values comprised between 0.752-0.811 N and skin break energy between 0.715-0.790 mJ for A and B ripening stages, respectively. Instead, Brancellao variety presented the softest skin with skin break force values ranged from 0.521 to 0.562 N and skin break energy from 0.407 to 0.475 mJ for A and B ripening stages, respectively. Ripeness grade increased with the berry cohesiveness for all the varieties studied.

Keywords: Texture parameters, Red varieties, Ripening stages, Varietal markers.

INTRODUCTION

One of the main factors involved in high quality wines is the ripeness degree of the grapes used for their elaboration. The evaluation of sugar/acidity ratio of the pulp is not sufficient to completely predict the oenological potential of red grapes for the elaboration of high quality wines.[1] In fact, phenolic compounds, extractable from grape skins and seeds, have a notable influence on the sensory properties of red wines such as colour, bitterness and astringency. In particular, highly important among red grapes is the gradual accumulation of anthocyanins in berry skins during ripening, being these class of phenolic compounds responsible of red colour of the grapes and the respective wines.[2,3] It was demonstrated that anthocyanin accumulation depended on several agroecological factors like cultivar, climatology and agronomical practices.[4-6]

The grapes richest in anthocyanins at harvest do not necessarily produce highly coloured wines. Anthocyanin extraction from the grape skin into the wine depends on the tendency of the berry skin to yield up them, as a consequence of the cellular wall degradation by pectolytic enzymes.[7,8] In this sense, it is important to consider that anthocyanin extractability increases through grape ripening as a consequence of the physiological changes occurred during grape berry development.

Several analytical methods have been proposed for the assessment of the phenolic ripeness grade in grapes. Nevertheless, those are generally rather complex and often require long analysis times.[9] Furthermore, the results interpretation can be difficult.[10] Recently, a mechanical property determined by texture test, namely skin break force, has proved to be able to estimate the anthocyanin extractability with adequate reliability.[11]

Texture Analysis is a current analytical technique usually used for measuring the physical properties of plant tissue.[12,13] Nevertheless, few scientific contributions have been published in relation to winegrape texture analysis.[14-16] The texture profile analysis (TPA) has been used for the textural evaluation of a wide range of food and vegetables.[17-20] Textural properties of whole berry depend on different characteristics like cell-wall composition, cell structure and pulp turgescence and, therefore, this mechanical test can be useful to follow grape ripening.[21]

A complete texture evaluation may be difficult due to the numerous parameters involved. There is a real risk of false conclusions being drawn or of results being misinterpreted when only partial characterization is carried out. In this sense, multivariate statistical analysis is a useful tool to elucidate influential parameters in grape texture analysis. Although berry skin break force and energy represent meaningful parameters for varietal characterization and differentiation,[22] only few previous works on grape texture measurements have investigated their efficiency for estimating grape quality through ripening[23-25] and someone reports the inability of different compression parameters to discriminate ripening stages.[21] Though much of this physico-mechanical knowledge was acquired on grapes sampled at different times during ripening, their physiological characteristics in a vineyard are very heterogeneous at any given date.[26]

In consequence, with the aim of verifying the real behaviour of texture parameters during ripening and their effective potentiality as varietal markers and ripeness predictors, this study was carried out with berries sampled at different advanced physiological stages. The sampling homogeneity was assessed by the content of total soluble solids.[27]

MATERIALS AND METHODS

Samples

Grape samples of three red cultivars (*Vitis vinifera* L.), namely Mencía (ME), Brancellao (BR) and Merenzao (MZ), were collected at different physiological stages from the same vineyard located in the Ribeira Sacra Denomination of Origin in 2008 year. This viticulture mountain area is located in Galicia (Northwest Spain) with a red wine production greater than 97 %. *Vitis vinifera* L. cv. Mencía is one of the most often used to produce quality red wines in Galicia. Brancellao and Merenzao varieties, considered native or of traditional culture in this region, represent less than 5 % of the total production but they are usually used like complement in winemaking. Furthermore, the vineyard re-structuring is being promoted, favouring the cultivation of Native varieties through the granting of economic aids financed with European funds.

Average age of grapevine is 15 years old. Each sample consisted of 500 grape berries, with pedicels, picked randomly up from ten different plants. Once picked berries were transported into the laboratory, these were separated according to their density (total soluble solids). Density was estimated by flotation of grape berries in different saline solutions (from 130 to 190 g/l sodium chloride) so that the difference in total soluble solids of two consecutive batches of berries was about 17 g/l (1 % potential alcohol).[27] Several ripening stages were studied: A (176 ± 8 g/l reducing sugars), B (193 ± 8 g/l reducing sugars), C (210 ± 8 g/l reducing sugars) and D (227 ± 8 g/l reducing sugars). The berries were visually inspected before analysis and those with damaged skins were discarded.

Instrumental Texture Analysis

A Universal Testing Machine TAxT2i□ Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) equipped with a HDP/90 platform and a 5 kg load cell was used for measuring mechanical parameters. All the data acquisitions were made at 400 Hz, involving the Texture Expert Exceed software version 2.54 for Windows. The operative conditions used in the different tests are shown in Table 1. Before the texture analysis, instrument was calibrated for force and distance.

A set of 20 berries was randomly sampled for each one of the several ripening stages defined by flotation. The puncture test was carried out on the bottom side (x), lateral side (y) and top side (z) of the berry.[28] Berry skin thickness measurement requires removing a piece of skin at the lateral side of each berry with care in removing the pulp from the skin and in positioning the skin sample on the platform to prevent folds in the skin.[22] Furthermore, it was convenient to insert an instrumental trigger threshold equal to 0.05 N that enabled the plane surface of the probe to adhere completely to the skin sample before the acquisition starts. It allowed a reduction or elimination of the 'tail' effect due to the postponement of the contact point.[29] Texture profile analysis (TPA) or double compression test was based on the analysis of each intact berry, which was compressed twice with a 25 % deformation, two second apart, in a reciprocating motion imitating the action of the jaw.[21,25,30] Test, pre-test and post-test speeds were 1 mm s^{-1}.[25] From the resulting force-time curve, the test extracted a number of textural parameters (Table 1).

Statistical analysis

All statistical analyses were performed using the SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

To facilitate the comprehension of the mechanical parameters measured, the typical force-time (or deformation) curves obtained by several texture tests are reported in Fig. 1. In particular, Fig. 1a shows a force-time (or deformation) curve achieved using the puncture test of berry skin. The berry skin hardness is assessed either by the maximum break force (F_{sk}) or by the break energy (W_{sk}). The first variable corresponds to the skin resistance to the probe penetration while the second variable is represented by the area under the curve, which is limited between 0 and F_{sk}. The curve related to berry skin thickness determination is reported in Fig. 1b. The berry skin thickness (Sp_{sk}) is given by the distance between the point corresponding to the probe contact with the berry skin (trigger, point 1) and the platform base HDP/90 (point 2). Fig. 1c represents a force-time curve corresponding to the two bite texture profile analysis. The variables A_1, A_{1W}, A_2 and A_{2W} correspond to the areas under compression and withdrawal portions of the first bite and second bite curve. The maximum first compression is defined by P_1, whereas d_2 represents the crosshead travel corresponding to the second compression. From them, BCo (berry cohesiveness), BG (berry gumminess), BCh (berry chewiness) and BR (berry resilience) were calculated according to the reported in Table 1.

Table 2 shows berry skin texture parameters for Mencía, Brancellao and Merenzao varieties at different ripening stages. No statistical difference was found among the different ripening stages studied for all grape varieties. Therefore, berry skin texture parameters could not be considered as good differentiating ripening stages. However, the berry skin break force and break energy for Brancellao variety were statistically lower than for Mencía and Merenzao varieties when the puncture test was carried out on the bottom side. Furthermore, the results obtained were significantly higher for Merenzao variety than for Brancellao and Mencía varieties when the puncture test was carried out on the lateral and top sides, except for the berry skin break energy found in the top side where similar values were obtained for Mencía variety at C ripening stage and for Merenzao variety at A ripening stage. There was no difference among varieties and ripening stages for the berry skin thickness and, therefore, this texture parameter did not permit to differentiate cultivars either. These results are consistent with data concerning berry skin thickness of the thirteen grape varieties studied in two consecutive vintages.[22] Thicker berry skin is not characterized by higher skin hardness as can be seen for all the varieties and ripening stages studied.

There is evidence of a significant relationship between the berry skin hardness measured on bottom, lateral and top sides and expressed as break force (Pearson coefficient=0.772-0.959, p=0.01) or break energy (Pearson coefficient=0.687-0.932, p=0.05). This implies a similar evolution of the skin hardness along ripening in the different berry sides.

It is important to take into account that berry skin hardness did not decrease with the increasing of total soluble solids as expected and in agreement with previously published data for different varieties cultivated in Italy.[25] Although the amount of the anthocyanins accumulated in the berry skin can be different for each ripening stage, they will be released at similar rate. A significant increase in Sp_{sk} values was instead found in Mondeuse grapes during on-vine drying process for the ice wines production. Moreover, a simultaneous increment of W_{sk} values was also observed in these overripe grapes.[29] In Brachetto and Nebbiolo varieties, grapes with higher skin break force produced, during a maceration in a model hydroalcoholic solution, extracts with higher content of anthocyanins, although they were released more slowly.[31]

Instrumental TPA parameters corresponding to different ripening stages of Mencía, Brancellao and Merenzao varieties are shown in Table 3. The values reported of berry cohesiveness were in good agreement for all the cultivars and ripening stages studied. So, they did not permit to establish a differentiation. On the other hand, a significant varietal effect was observed for berry hardness, gumminess, springiness, chewiness and resilience on Merenzao variety. Thus, Merenzao had the highest hardness, gumminess, springiness and chewiness but the least resilient berries. Moreover, a decreasing tendency was found for the parameters determined in all varieties when ripeness increased. So, some relevant differences (higher than 6 %) were observed among ripening stages for each variety. For Mencía, berry springiness and resilience showed lower values at C ripening stage. For Brancellao, berry hardness and gumminess reported higher values at A ripening stage. For Merenzao, lower values of berry hardness, gumminess, springiness and chewiness corresponded to

D ripening stage, whereas higher values of berry resilience corresponded to A ripening stage. In addition, berry hardness and chewiness showed lower values at C and B ripening stage than the former ones (A, B and A, respectively).

In an attempt to differentiate grapes according to variety and ripening stage, and to know the most markedly influential texture parameters on the sample similarities and differences, the results obtained were subjected to principal component analysis (PCA). Three principal components explained 90.0 % of the variability in the original data. Two-dimensional diagram, where the first principal component is plotted against the third principal component, is shown in Fig. 2. The first principal component accounted for 70.9 % of the total variance, which grouped only the samples according to variety. Although this first component is mainly associated with the berry skin break force and energy measured on the lateral side, other texture parameters being ones measured on the top side, berry chewiness and berry resilience. The third principal component accounted for 8.11 % of the total variance, which grouped the samples according to ripening stage. Component 3 is mainly associated with berry cohesiveness. The loadings of each variable obtained from PCA can be seen in Table 4. So, the highest values of the third principal component corresponded to the most ripeness grapes.

The berry skin break force and energy measured on the lateral side are meaningful mechanical variables for varietal characterization and differentiation. It is consistent with the data previously reported for Galician and Italian varieties.[22,25] Furthermore, berry chewiness is also a dominant texture parameter in differentiating Italian varieties,[25] but there are no data previously published about texture profile parameters in Galician varieties. On the other hand, our results verified the ability of several texture parameters to differentiate both varieties and ripening stages, this finding being not in good agreement with that reported by other authors.[21] In the last work, no correct classification was observed, even when the analyses were performed in each one of the different parcels studied. However, mechanical texture parameters were able to show differences between grapes having different ripening level and some of them permitted the origin of the grapes to be distinguished.[30] The worst classified sample was Merenzao variety at B ripening stage because its value of principal component 3 is similar than that corresponding to Brancellao variety at A ripening stage.

CONCLUSIONS

The results obtained show that a clear differentiation among varieties and ripening stages was possible using texture parameters. The most influential parameters on variety differentiation were the berry skin break force and energy measured on the lateral side. The hardest berry skin was associated with Merenzao variety, whereas Brancellao variety presented the softest skin. Regarding to ripeness differentiation, berry cohesiveness permitted the consistent classification of the grapes analyzed. Ripeness grade increased with berry cohesiveness. The berry skin break force and energy measured on the lateral side were statistical correlated with all the texture profile parameters, with exception of berry cohesiveness. Once the usefulness of these texture indices as ripening stage markers has been verified, further studies are necessary to assure their evolution during the grape development and to establish a predictive model. It could facilitate the harvest date prediction and the winemaking management.

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FIGURE CAPTIONS

Figure 1 Typical curves corresponding to berry skin hardness test (a), berry skin thickness test (b) and the two bite texture profile analysis (c).

Figure 2 Principal component analysis of Mencía, Brancellao and Merenzao varieties for different ripening stages according to the first and third components.

Table 1 Operative conditions for the measurement of the berry textural characteristics

Test	Probe	Test speed (mm/s)	Compression (mm)	Mechanical property	References
Skin hardness	P/2N, 2 mm needle	1	3	F_{sk} = berry skin break force (N) W_{sk} = berry skin break energy (mJ)	[28]
Skin thickness	P/2, Ø 2 mm	0.2	–	Sp_{sk} = berry skin thickness (μm)	[22] [29]
Texture Profile Analysis	P/35, Ø 35 mm	1	25 % deformation	BH = berry hardness (N): force corresponding to P_1 (force necessary to attain a given deformation) BCo = berry cohesiveness: $(A_2+A_{2W})/(A_1+A_{1W})$ (strength of internal bonds making up berry body) BG = berry gumminess (N): BH*BCo (force necessary to disintegrate a semisolid food until ready for swallowing) BS = berry springiness (mm): D_2 (distance recovered by sample during time comprised between the end of first bite and the start of second bite) BCh = berry chewiness (mJ): BH*BCo*BS (energy necessary to chew a solid food until ready for swallowing) BR = berry resilience: (A_{1W}/A_1) (how well berry fights to regain original position)	[25] [21] [30]

Table 2 Berry skin texture parameters for Mencía, Brancellao and Merenzao varieties at different ripening stages

Variety	Ripeness	Fx_{sk} (N)	Wx_{sk} (mJ)	Fy_{sk} (N)	Wy_{sk} (mJ)	Fz_{sk} (N)	Wz_{sk} (mJ)	Sp_{sk} (μm)
Mencía	A	0.466±0.102a	0.381±0.109a	0.584±0.136a	0.556±0.178a	0.387±0.073a	0.279±0.099a	257±49a
Mencía	B	0.475± 0.093a	0.389±0.116a	0.574±0.116a	0.509±0.141a	0.359±0.101a	0.258±0.111a	258±46a
Mencía	C	0.498± 0.100a	0.413±0.137a	0.610±0.106a	0.549±0.120a	0.437±0.115a	0.340±0.158a,b	238±32a
Brancellao	A	0.344±0.054b	0.225±0.053b	0.521±0.148a	0.407±0.138a	0.398±0.107a	0.279±0.121a	240±52a
Brancellao	B	0.331±0.067b	0.192±0.056b	0.562±0.123a	0.475±0.150a	0.346±0.108a	0.231±0.108a	234±57a
Brancellao	C	0.377±0.060b	0.227±0.072b	0.532±0.108a	0.415±0.132a	0.368±0.062a	0.235±0.064a	266±57a
Merenzao	A	0.496±0.117a	0.374±0.145a	0.752±0.178b	0.715±0.245b	0.537±0.119b	0.410±0.143b,c	234±56a
Merenzao	B	0.530±0.101a	0.411±0.122a	0.811±0.158b	0.790±0.244b	0.597±0.101b	0.493±0.151c	248±59a
Merenzao	C	0.545±0.106a	0.421±0.137a	0.756±0.148b	0.724±0.218b	0.585±0.108b	0.483±0.153c	233±41a
Merenzao	D	0.517±0.104a	0.396±0.127a	0.757±0.134b	0.727±0.183b	0.591±0.143b	0.515±0.216c	240±50a

All data are expressed as average value ± standard deviation (n=20). Different letters within the same column indicate significant differences (*Tukey-b test*; α=0.05). A: 176 ± 8 g/l reducing sugars; B: 193 ± 8 g/l reducing sugars; C: 210 ± 8 g/l reducing sugars; D: 227 ± 8 g/l reducing sugars; F_{sk}: berry skin break force; W_{sk}: berry skin break energy; x: bottom side; y: lateral side; z: top side; Sp_{sk}: berry skin thickness.

Table 3 Texture profile analysis parameters for Mencía, Brancellao and Merenzao varieties at different ripening stages

Variety	Ripeness	BH (N)	BCo	BG (N)	BS (mm)	BCh (mJ)	BR
Mencía	A	3.35±0.50a	0.705±0.074a	2.37±0.48a	2.14±0.19a,b,c	5.14±1.31a	0.400±0.050a
Mencía	B	3.36±0.64a	0.726±0.034a	2.43±0.43a	2.05±0.20a,b	5.01±1.21a	0.376±0.029a
Mencía	C	3.29±0.57a	0.709±0.031a	2.32±0.35a	1.90±0.14a	4.42±0.85a	0.351±0.022b
Brancellao	A	3.70±0.90a,b	0.715±0.063a	2.65±0.69a,b	1.91±0.50a	5.18±2.27a	0.388±0.035a
Brancellao	B	3.33±0.75a	0.734±0.029a	2.44±0.53a	1.99±0.30a	4.98±1.66a	0.384±0.029a
Brancellao	C	3.33±0.65a	0.745±0.031a	2.48±0.49a	1.91±0.32a	4.84±1.58a	0.400±0.030a
Merenzao	A	4.72±1.10c	0.712±0.070a	3.33±0.73c	2.39±0.24c	8.07±2.45b	0.340±0.020b
Merenzao	B	4.57±0.99c	0.700±0.052a	3.18±0.67b,c	2.31±0.28b,c	7.45±2.18b,c	0.313±0.022c
Merenzao	C	4.26±1.17b,c	0.736±0.053a	3.10±0.74b,c	2.29±0.29b,c	7.25±2.56b,c	0.326±0.022b,c
Merenzao	D	3.79±1.07a,b	0.742±0.041a	2.81±0.81a,b,c	2.15±0.34a,b,c	6.27±2.59a,c	0.327±0.025b,c

All data are expressed as average value ± standard deviation (n=20). Different letters within the same column indicate significant differences (*Tukey-b test*; α=0.05). A: 176 ± 8 g/l reducing sugars; B: 193 ± 8 g/l reducing sugars; C: 210 ± 8 g/l reducing sugars; D: 227 ± 8 g/l reducing sugars; BH: berry hardness; BCo: berry cohesiveness; BG: berry gumminess; BS: berry springiness; BCh: berry chewiness; BR: berry resilience.

Table 4 Loadings of each variable in the three principal components

	Component		
Variable	1	2	3
Fx_{sk}	0.820	0.532	0.141
Wx_{sk}	0.712	0.669	0.063
Fy_{sk}	0.984	0.011	0.088
Wy_{sk}	0.974	0.116	0.060
Fz_{sk}	0.958	-0.040	0.184
Wz_{sk}	0.941	0.037	0.250
Sp_{sk}	-0.465	0.454	-0.107
BH	0.888	-0.311	-0.289
BCo	-0.221	-0.379	0.815
BG	0.884	-0.379	-0.172
BS	0.886	-0.066	-0.238
BCh	0.915	-0.292	-0.181
BR	-0.928	-0.033	-0.212

F_{sk}: berry skin break force; W_{sk}: berry skin break energy; x: bottom side; y: lateral side; z: top side; Sp_{sk}: berry skin thickness; BH: berry hardness; BCo: berry cohesiveness; BG: berry gumminess; BS: berry springiness; BCh: berry chewiness; BR: berry resilience.

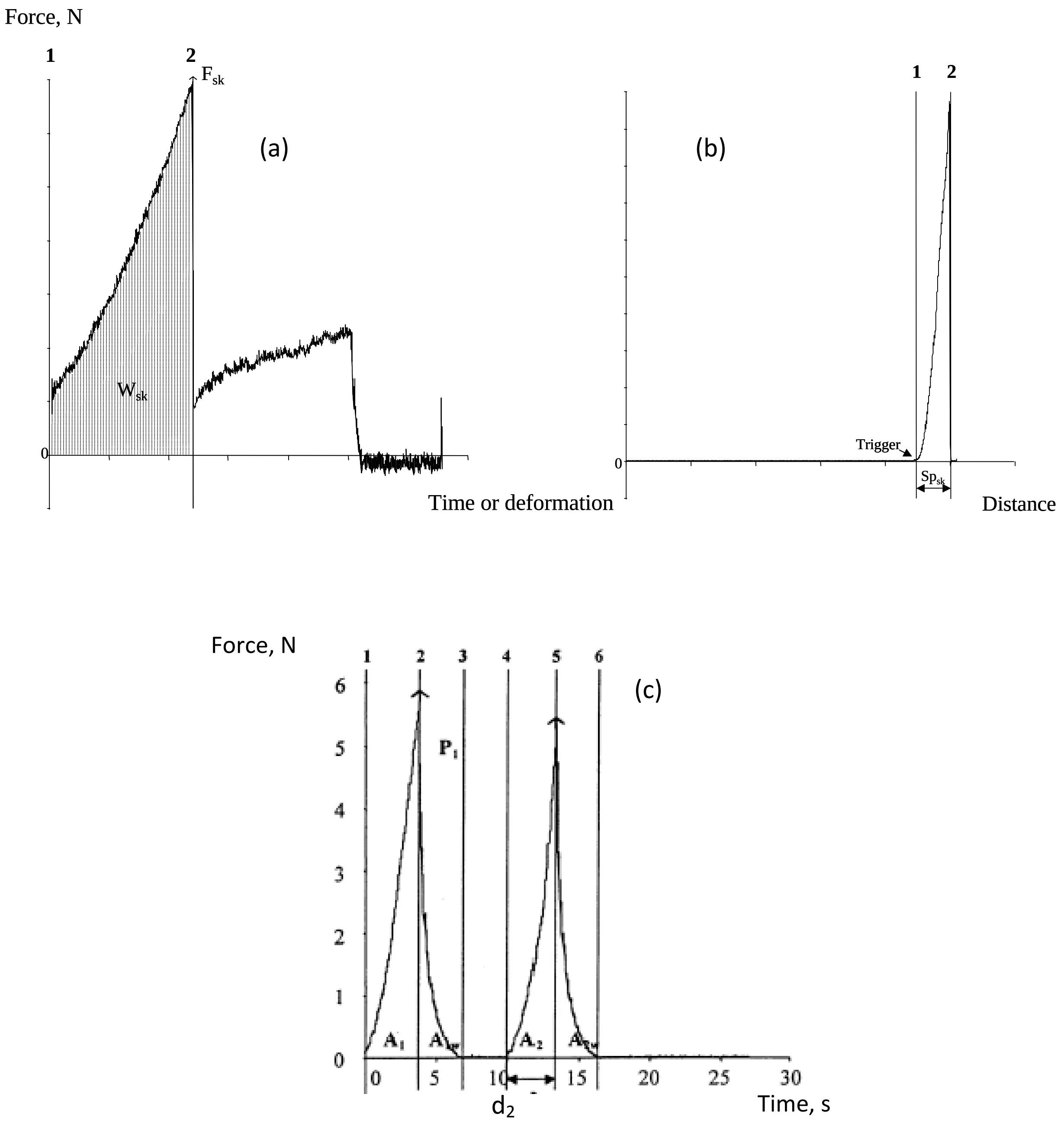
Force, N
1
2
F_sk
(a)
W_sk
0
Time or deformation
1
2
(b)
Trigger
0
Sp_sk
Distance
Force, N
1 2 3 4 5 6
6
5
4
3
2
1
0
(c)
P_1
A_1
A_1w
A_2
A_2w
0 5 10 15 20 25 30
d_2
Time, s

Figure 1

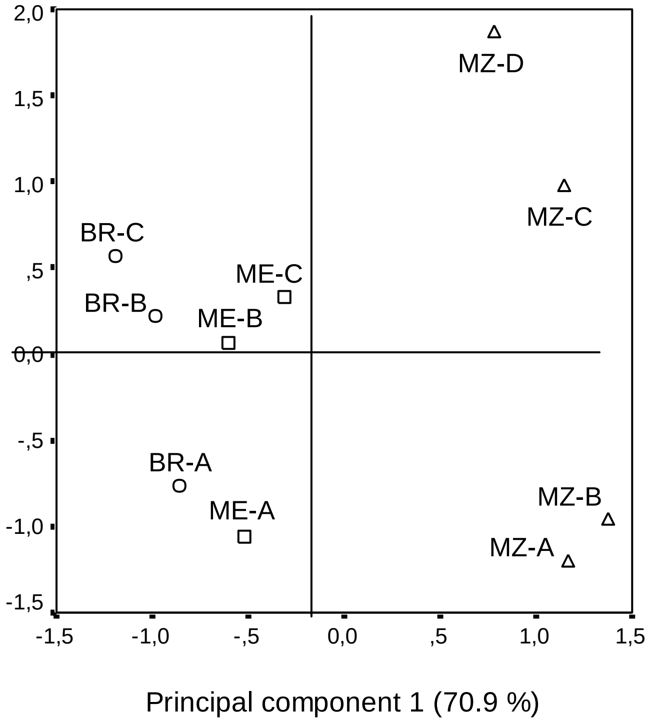
2,0
1,5
1,0
,5
0,0
-,5
-1,0
-1,5
MZ-D
MZ-C
BR-C
ME-C
BR-B
ME-B
BR-A
ME-A
MZ-B
MZ-A
-1,5
-1,0
-,5
0,0
,5
1,0
1,5
Principal component 1 (70.9 %)

Figure 2